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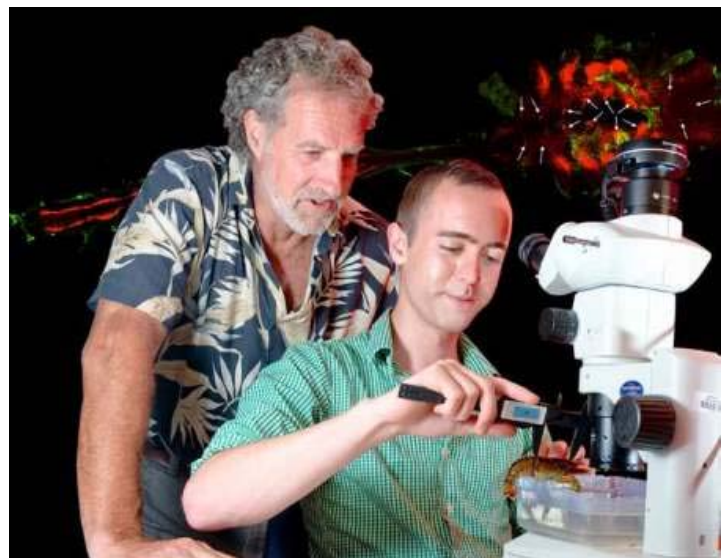
Feature Articles

Beyond the Claw Crackers: Investigating a Neurobiological Bridge between the Environment and Behavioral Rhythms in Juvenile American Lobsters

—Brigid C. Casellini

While some in the greater University of New Hampshire community are most concerned with the culinary aspect of lobsters, others are hard at work figuring out just what makes these desirable crustaceans tick. Cody White, a senior neuroscience major, has devoted the latter part of his undergraduate career to studying the complex nervous system of the juvenile American lobster in a laboratory on the Durham campus.

As happens for many undergraduate researchers, Cody's project started relatively small and evolved over the course of two years. In the end, he and his mentor, Professor of Zoology Win Watson, demonstrated that juvenile lobsters express daily rhythms of activity, with a preference for being most active at night. By observing that this rhythm persists in the absence of environmental light signals, they proved the existence of an endogenous circadian clock. Much more groundbreaking, however, was their use of bioinformatics software to identify a gene that coded for the clock protein, cryptochrome, and the use of a staining technique, immunohistochemistry, to identify the neurons in the lobster central nervous system that contain the cryptochrome protein. Cryptochrome is an integral contributor to the molecular actions that clock cells carry out, which ultimately influence behavior. When clock genes are expressed or inhibited, different locomotor behaviors result. This is a characteristic of clock cells that is shared among many animals, including humans.



Cody White (right) and his mentor, Professor Win Watson

Cody's experiments involved, among other things, twelve lobsters, an aquarium, blindfolds, cameras, and video analysis software. What follows is our conversation with Cody. He explains how he got involved in undergraduate research, how his experiments evolved, and what he hopes to do in the future.

Inquiry: You received an Undergraduate Research Award (URA) from the Hamel Center for Undergraduate Research in summer 2012, but your research started before then with some preliminary experiments. Please explain what attracted you to this topic and how you became involved in Professor Watson's laboratory.

CW: I started working in Professor Watson's lab in September of 2011. He had sent out an email to neuroscience majors describing research positions that were available in his lab. The position I found appealing involved the use of immunohistochemistry, a cell staining technique used to visualize the presence of proteins, hormones, and

neurotransmitters in tissues, like the nervous system. Dr. Watson wanted to map the circuits of the nervous system of several marine organisms that are involved in circadian locomotor rhythm regulation. He was also looking for someone to pursue genetics research on this topic. I had prior experience in immunohistochemistry, an interest in genetics, and a fascination with neural circuitry, so I immediately introduced myself and told him about my interest in the position. We started working together soon after that.

Inquiry: Your summer research focused on determining the presence of photosensitive neurons in the abdominal ganglion of juvenile lobsters and whether these neurons were capable of entraining lobsters to the light/dark cycle. Please tell us the basics of your experiment.

CW: First, we had to prove that lobsters express a circadian rhythm of activity and inactivity. UNH research divers supplied us with lobsters they caught off the coast of NH, and the New England Aquarium donated lobsters as well. They were held in an aquarium and exposed to a natural summer light:dark cycle, fourteen hours light/ten hours dark. To initiate an experiment, three lobsters were then moved to separate containers where they were exposed to varying environmental light stimuli. Their movement was recorded with a low-light camera and time-lapse software, and then analyzed with video analysis software called Ethovision. This was repeated four times for a total of twelve lobsters.

Once the circadian rhythm had been established, we then blindfolded the lobsters to see if they would still express circadian rhythm, based on the assumption that photoreceptors in the abdominal ganglia were also capable of entraining the lobster to the light/dark cycle. Finally, to test for the presence of cryptochrome, we excised the ventral nerve cords of sixteen lobsters and used immunohistochemistry to determine the protein's presence. Because an organism's cryptochrome levels are thought to fluctuate throughout the day, I dissected four lobsters at noon, four at midnight, four at sunrise, and four at sunset. After staining the nerve cords with primary antibodies directed at cryptochrome, we added fluorescent-labeled secondary antibodies that bound to the primary antibodies. Confocal microscopy, a powerful imaging tool that can scan through the depth of the tissue, allowed us to visualize the neurons that fluoresced, and, therefore, contained cryptochrome.

Inquiry: Were your actual responsibilities in the lab as you expected, and did you have the opportunity to work with others?

CW: My responsibilities were generally the same as expected, but the number of experiments that we could produce was large enough that other students, like Scott Finnance and Kyle Harris, became involved too. We gained considerable evidence over the summer that supported our hypothesis, that the light sensitive protein, cryptochrome, was localized in specific neurons of the abdominal ganglia and in the lobster's genome. So the three of us worked both as a team and independently with the common goal of furthering our understanding of the possible neurons involved in regulating the lobster's circadian rhythms.

Inquiry: How did your research evolve over the course of the summer and into the fall? Did it morph into something else based on your discoveries and the technology available to you?

CW: We've expanded the project and are collaborating with Dr. Chris Chabot and some of his students at Plymouth State University. We thought we had found the blue light photoreceptor form of cryptochrome; however, further genomic analyses revealed the cryptochrome we found did not possess photoreceptor abilities. Instead, cryptochrome-2 was its identity, which means it still regulates the circadian clock cells' cycle but is activated downstream of photoreceptors by chemical means. Based on our team finding most of the cryptochrome-2 gene and using immunohistochemistry to visualize the neurons containing the cryptochrome-2 protein, we have expanded our experiment in a couple of ways. The team is now working with the Hubbard Gene Sequencing Center at UNH to complete the genetic and protein sequences of the cryptochrome-2 we detected. We are also working toward demonstrating that the amount of cryptochrome-2 protein fluctuates over a twenty-four hour period, as is common among proteins involved in the molecular circadian clock cells; to do this we are employing a quantitative technique called western blots. Dr. Charles Walker, some of his students, and Dr. Thomas Foxall were helpful in getting us up and running with the western blots. With the inter-college team's continued collaborative work, I am confident that we will soon have a very detailed description of the American lobster's cryptochrome-2, including its gene's full identity, its amino acid sequence, how and if the protein levels fluctuate in a temporal manner, and where the proteins are localized in the animal's nervous system.

This all touches on a bigger picture though; that is, the cryptochrome-2 containing neurons are part of the neural circuitry that may influence the lobster's nocturnal activity and diurnal inactivity. This could lead to many future chronobiology experiments using the American lobster as a model organism. If the live nervous system is removed from the lobster and kept alive in a dish, we can use a technique known as electrophysiology to record the electrical rhythms of the clock cells throughout exposure to a twelve hour light/twelve hour dark cycle. In doing so, we can begin to understand how the cells send signals to the musculature, ultimately influencing behavior. We could then

correlate the light/dark signals, the rhythms of the clock cells (protein expression levels and electrical rhythms), and the activity or inactivity of the lobster. Understanding all of these things leads toward understanding how the neural circuitry involving the clock cells and photoreceptors of the eyes influence behavioral rhythms.

These data may then be translatable to understanding the mechanisms that the human clock cells, in the hypothalamus, use to influence our behavioral rhythms. Most understanding of how the human nervous system operates came from experiments on animals; for instance, studies on the giant squid's nervous system led to understanding of how electrochemical signals are transmitted through long, large nerves. Chronobiological studies on the American lobster may reveal the lobster to be a promising candidate model for understanding how the clock cells regulate behavioral rhythms.

Inquiry: What excites you most about this research topic?

CW: I'm excited to learn that the biology of organisms, sometimes thought by people to be "lower" or "insignificant" in comparison to humans, is incredibly complex. From what research and the literature have revealed, all animals have internal clocks that regulate their behaviors in response to the twenty-four hour light/dark cycle. From what I understand, though, people don't know much about the link between these circadian clock cells and the behavior that they regulate. Yes, scientists can manipulate the expression of a clock cell's genes and see that the behavior of the animal changes—a strategic cause-and-effect sort of approach. Yet, I am interested in the biochemical *links* between sensation of environmental stimuli (e.g., photo-sensation), neural clock cell processing, and elicitation of behavior. What really excites me is learning about the dynamics of the signals coming from the environment; how they catalyze molecular phenomena in the clock cells, which send complex messages throughout the nervous system and musculature, resulting in the organism expressing a complex behavior.

Inquiry: This will serve as the basis of your senior thesis, correct? Do you anticipate continuing this line of research as a grad student at UNH or elsewhere?

CW: Yes, this will serve as my senior thesis project. We hope to pitch an article about our work in the near future detailing our identification of cryptochrome-2 in the American lobster nervous system. We have not yet decided which scientific journal we will pitch it to, but articles on chronobiology have been featured in a wide range of journals, including *Chronobiology* and *Cell*. I have applied to the UNH graduate program as well as graduate programs at other universities. I want to see what options are available to me, but if I do choose to stay at UNH, then I will definitely continue to pursue this research with Dr. Watson.

Inquiry: Is there anything else you would like to share with our readers, especially those undergraduates who are considering becoming involved in research?

CW: The research experiences I have had at UNH have been the highlight of my academic experience and are a great stepping-stone for future professional endeavors. Learning skills and experimental techniques from UNH mentors, such as Win Watson, Thomas Foxall, Charles Walker, and Mark Townly, has given me the opportunity to not only express myself creatively through scientific experimentation but has also instilled in me an enormous appreciation for understanding life. I would like to thank these brilliant mentors for the guidance they've given me throughout my time as an undergraduate. I would urge students at UNH and at other universities to seek out research opportunities because undergraduate research turned out to be the best thing I have ever pursued.

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